Anaerobic Degradation of Benzene in Diverse Anoxic Environments

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Benzene has often been observed to be resistant to microbial degradation under anoxic conditions. A number of recent studies, however, have demonstrated that anaerobic benzene utilization can occur. This study extends the previous reports of anaerobic benzene degradation to sediments that varied with respect to contamination input, predominant redox condition, and salinity. In spite of differences in methodology, microbial degradation of benzene was noted in slurries constructed with sediments from various geographical locations and range from aquifer sands to fine-grained estuarine muds, under methanogenic, sulfate-reducing, and iron-reducing conditions. In aquifer sediments under methanogenic conditions, benzene loss was concomitant with methane production, and microbial utilization of [^{14}C]benzene yielded $^{14}CO_2$ and $^{14}CH_4$. In slurries with estuarine and aquifer sediments under sulfate-reducing conditions, the loss of sulfate in amounts consistent with the stoichiometric degradation of benzene or the conversion of [^{14}C]benzene to $^{14}CO_2$ indicates that benzene was mineralized. Benzene loss also occurred in the presence of Fe(III) in sediments from freshwater environments. Microbial benzene utilization, however, was not observed under denitrifying conditions. These results indicate that the potential for the anaerobic degradation of benzene, which was once thought to be resistant to non-oxygenase attack, exists in a variety of aquatic sediments from widely distributed locations.

Introduction

Benzene is a naturally occurring aromatic compound present in petroleum fuels and is a frequent contaminant in both surface and subsurface environments. It is also fairly water soluble, toxic, and carcinogenic (*1*), thus its transport and fate in the environment has attracted a considerable amount of regulatory scrutiny. Aerobic microorganisms readily oxidize benzene to carbon dioxide (*2, 3*), thus benzene does not typically persist in oxic environments. Many aquifers and aquatic sediments, however, have extensive anoxic zones in which denitrification, Fe(III) reduction, sulfate reduction, or methanogenesis predominates (*4*). Although microbial

utilization of toluene (*5–9*), ethylbenzene (*10*), and xylenes (*6, 11–14*) under various anoxic conditions has been well-documented (as reviewed in ref 15), biodegradation of benzene has been inconsistently observed in the absence of oxygen. In fact, a number of reports have shown that benzene resists anaerobic metabolism in the field (*16, 17*) and in laboratory enrichments established with sewage sludge, groundwater sediments, and contaminated soils (*15, 17–20*). Furthermore, in several studies, low dissolved oxygen concentrations were often associated with a diminution in benzene utilization (e.g., refs 12, 17, and 21).

Benzene degradation in the absence of oxygen was noted as early as 1980, when Ward et al. (*22*) reported the formation of small amounts (up to 2%) of $^{14}CO_2$ and $^{14}CH_4$ from [^{14}C]-benzene in methanogenic enrichments derived from petroleum-contaminated salt marsh and estuarine sediments. Microbial metabolism of benzene to carbon dioxide and methane has been observed under methanogenic conditions in enrichment cultures from sewage sludge (*23*), and benzene mineralization to CO_2 has been observed in mesocosms containing river sediments (*24*). In enrichment cultures with sewage sludge, ^{18}O from [^{18}O]H$_2$O was incorporated into benzene as a hydroxyl group with the formation of phenol (*9*). A pathway for anaerobic benzene metabolism was proposed in which the phenol was subsequently converted to cyclohexanone before complete degradation to CO_2 and CH_4 (*23*). Only a minor portion (<6%) of the [^{14}C]benzene added to the cultures, however, was converted to $^{14}CO_2$. Benzene was also consumed in methanogenic aquifer material incubated under anoxic conditions, but it was not determined whether the benzene was mineralized to carbon dioxide and methane (*25*).

More recent studies indicate that, under appropriate conditions, benzene can be oxidized to CO_2 in the absence of oxygen with either sulfate or Fe(III) serving as the electron acceptor. For example, rapid benzene mineralization under sulfate-reducing conditions in marine and freshwater sediments and in aquifer material have been reported (*26–31*). In instances in which the stoichiometry of benzene consumption and sulfate loss has been determined, the results have been consistent with sulfate serving as the electron acceptor for benzene oxidation (*27, 28, 31*). Benzene mineralization was also observed in sediments taken from the Fe(III)-reduction zone of a petroleum-contaminated aquifer and amended with synthetic Fe(III) chelators or humic acids (*32, 33*). The stoichiometry of benzene metabolism and Fe(III) reduction indicated that Fe(III) was the sole electron acceptor for benzene oxidation in these sediments (*32*).

Evidence for the microbial degradation of benzene under denitrifying conditions is less pursuasive. Although nitrate-dependent uptake of benzene was observed in aquifer sands or groundwater incubated under anoxic conditions (*34, 35*), neither of these studies demonstrated the oxidation of benzene to CO_2 or established stoichiometric relationships between benzene metabolism and nitrate loss. Other studies have investigated the potential for benzene oxidation coupled to nitrate reduction and have found that benzene persists under denitrifying conditions (*11–13, 17*).

In the study reported here, the potential for benzene degradation was examined in a diversity of sedimentary environments obtained from various locations and examined in different laboratories. The fact that anaerobic benzene degradation was observed despite differences in sites, methodologies, and analytical techniques underscores the strength of the observations. The results demonstrate that, for the first time, [^{14}C]benzene can be effectively mineralized to $^{14}CO_2$

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TABLE 1. Characteristics of Samples Assayed for Presence of Microorganisms Capable of Anaerobic Benzene Degradation and Reference to Procedures Used in Assay Protocol

sample type	location	site characteristics	redox condition at site[a]	assay conditions[a]	assay procedure[b]
sediment near landfill	Oklahoma	aquifer contaminated with landfill leachate	M	M, S	43
sediment from Sleeping Bear Dunes National Park	Michigan	aquifer contaminated with gasoline	M	M, S, N	43
sediment from Seal Beach	California	aquifer contaminated with gasoline	S	S	43
New York/New Jersey Harbor	New York/New Jersey	estuary contaminated with petroleum	S	M, S, I, N	this study
Potomac River	Maryland	river sediments	I	I	38

[a] Redox and assay conditions: M, methanogenic; S, sulfate-reducing; I, iron-reducing; N, denitrifying. [b] Literature citation to detailed anaerobic biodegradation assay procedure.

and $^{14}CH_4$ under methanogenic conditions and substantiate the potential for anaerobic benzene oxidation under sulfate-reducing and Fe(III)-reducing conditions.

Materials and Methods

Site Descriptions. Sediment samples were obtained from several sites that varied with respect to contamination history, predominant redox condition, and salinity. Complete descriptions of the sites can be found in references cited in Table 1 or are summarized below. Table 1 also includes an indication of the assay conditions under which benzene utilization was evaluated.

Collection and Characterization of Inocula. Sediments used as inocula were collected as previously described from a variety of locations including the following: (i) a shallow, anoxic aquifer polluted by leachate from the municipal landfill in Norman, OK (36); petroleum-contaminated aquifers at (ii) the Sleeping Bear Dunes National Lakeshore near Empire, MI (37), and (iii) Seal Beach, CA (26). Sediments were also collected from (iv) the New York/New Jersey Harbor (NY/NJ) near the Fresh Kills landfill and (v) the Potomac River, MD (38). Sediments from NY/NJ Harbor were taken using a gravity corer with a PVC-lined sleeve (6.5 cm i.d.) from the surficial 30–40 cm of sediment. The sediment cores were capped immediately after collection and placed on ice for transport back to the lab. The cores were kept at 4 °C until use in the experiments. All sites were chronically exposed to fuel hydrocarbons except the leachate-contaminated aquifer in Norman, OK, and the Potomac River, MD. The sampling sites were previously characterized as methanogenic (i and ii), sulfidogenic (iii and iv), or Fe(III)-reducing (v).

Anaerobic Biodegradation Assays. The incubations were designed to assess benzene metabolism under methanogenic, sulfate- or Fe(III)-reducing, or denitrifying conditions. Procedures for the biodegradation assays are referenced in Table 1 or are described below. Evidence for microbial utilization of benzene was based on (1) the disappearance of the substrate and its conversion to metabolic end products including methane and carbon dioxide or (2) the production of Fe^{2+} or the loss of nitrate or sulfate, when ferric iron or the latter anions were included in the sediment slurries as terminal electron acceptors. The resulting information was interpreted relative to both sterile and benzene-unamended controls. The experimental bottles were made in at least triplicate.

Aquifer Sediments from OK, MI, and CA. The sediment slurries were constructed in an anaerobic glovebox under N_2:H_2 (90:10). The slurries consisted of 50 ± 1 g of sediment and 75 ± 1 mL of groundwater or media (see below) added to sterile 160-mL serum bottles. Sterile Na_2S (1 mM) and resazurin (0.0001%) were added as a reductant and a redox indicator, respectively. The bottles were closed with a composite stopper made of the top of a butyl rubber stopper fused to the bottom of a Teflon-coated stopper. The bottles were then removed from the glovebox, and the closures were held in place with aluminum crimp seals. The headspace of the vials was exchanged three times with N_2:CO_2 (80:20). Sterile controls were obtained by autoclaving the slurries on three successive days. In experiments where sulfate or nitrate served as the terminal electron acceptor, slurries were amended with sulfate or nitrate from sterile, anoxic stock solutions to a concentration of 20 mM. Due to sample constraints, we substituted a basal medium (39) to construct slurries with MI or CA sediments in experiments where sulfate served as the terminal electron acceptor. The basal medium was modified by omitting the cysteine hydrochloride and by adding 5 mL L^{-1} of a vitamin mixture (40). Slurries of CA sediments were constructed with either the modified basal medium or a mineral salts medium (26).

Undiluted benzene was added to achieve concentrations of 1.4–4.3 mM in the experiment with OK sediments. In one study with MI sediments, benzene was introduced at a concentration of 675 μM. In this experiment, benzoate was added to some bottles to serve as a positive control for methanogenesis. Benzoate was introduced from a sterile, anoxic stock solution to achieve a carbon concentration of 50 ppm, which was equivalent to the amount of carbon in the benzene-amended bottles. For radiolabel studies with MI and CA sediments, [^{14}C-UL]benzene (Sigma Chemical Co., St. Louis, MO; >98% purity; specific activity of 53.4 or 63.2 mCi mmol^{-1}) in an anoxic stock solution of unlabeled benzene (7.5 mM) was added to the bottles. The radioactivity was 3.5–7.0 × 10^6 dpm (7.0–9.3 × 10^4 dpm mL^{-1}), and the total benzene concentration was approximately 50 μM in each bottle. All incubations were carried out at room temperature (22 °C) in the dark.

Estuarine Sediments from NY/NJ Harbor. Sediment slurries (10:90; vol sediment:vol media) were prepared in the same manner as previously described (41), except that the 50-mL serum bottles were capped with Teflon-coated, butyl rubber stoppers (Emsco, Philadelphia, PA) and crimp-sealed. Media used to slurry the sediments were prepared using standard anaerobic techniques. Each liter of basic medium contained 1.3 g of KCl, 0.2 g of KH_2PO_4, 23 g of NaCl, 0.5 g of NH_4Cl, 0.1 g of $CaCl_2 \cdot 2H_2O$, 1.0 g of $MgCl_2 \cdot 6H_2O$, 2.5 g of $NaHCO_3$, 0.1 mg of resazurin, 10 mL of vitamin stock, and 15 mL of trace salts solution. Each liter of trace salts stock contained 30 mg of $CoCl_2 \cdot 6H_2O$, 0.15 mg of $CuCl_2$, 5.7 mg of H_3BO_3, 20 mg of $MnCl_2 \cdot 4H_2O$, 2.5 mg of $Na_2MoO_4 \cdot 2H_2O$, 1.5 mg of $NiCl_2 \cdot 2H_2O$, and 2.1 mg of $ZnCl_2$. For each reducing condition, 1 L of basic medium was amended with the following: 0.368 g of $FeCl_2 \cdot 4H_2O$ and 0.5 g of $Na_2S \cdot 9H_2O$ for methanogenic medium; 2.84 g of Na_2SO_4, 1.49 mg of $FeCl_2 \cdot 4H_2O$, and 0.35 g of $Na_2S \cdot 9H_2O$ for sulfidogenic medium; freshly precipitated amorphous Fe floc for Fe(III)-reducing medium; 3.3 g of KNO_3 and 1.49 mg of $FeCl_2 \cdot 4H_2O$ for denitrifying medium. The initial concentration of electron acceptors was 20 mM sulfate, 200 mM Fe(III), and 30 mM nitrate for sulfidogenic, Fe(III)-reducing, and denitrifying media, respectively. Benzene was added to the incubation mixtures to an initial concentration of 125 μM. All incubations, with autoclaved controls, were incubated without shaking in the dark at 30 °C.

Freshwater Sediments from MD. The brown, oxidized layer of sediment from the Potomac River was used to construct slurries (100 mL) and transferred under N_2:CO_2 (93:7) into

TABLE 2. Evidence for Anaerobic Biodegradation of Benzene in Samples from Diverse Environments under Different Redox Conditions

sample type	redox condition[a]	benzene concentration (μM)			initial lag time (d)	incubation time (d)	comments
		initial	final	corrected[b]			
aquifer sediment from a national park, MI	M	50	16.5	34	420	520	$^{14}CH_4$ and $^{14}CO_2$ detected (Table 3)
	M	675	a[c]		360	590	CH_4 observed (Figure 1)
	M control	50	54				
	S	50	0	40	~400	500	$^{14}CO_2$ detected (Table 3)
	S control	50	40				
	N	50	51	NC[e]		530	
	N control	50	53				
aquifer sediment from Seal Beach, CA	S	57	5	22	120	320	$^{14}CO_2$ detected (Table 3)
	S control[d]	68	38				
New York/New Jersey Harbor	M	125	0	55	100	180	
	M control	125	55				
	S	125	0	86	60	100	coupled to SO_4^{2-} reduction (Table 4)
	S control	125	86				
	I	125	0	52	100	180	
	I control	125	52				
	N	125	40	NC		210	
	N control	125	35				
Potomac River	I	3	0	3	50	60	
	I	3	3				

[a] Redox and assay conditions: M, methanogenic; S, sulfate-reducing; I, iron-reducing; N, denitrifying. [b] Corrected refers to benzene loss in cultures above loss in sterile controls. [c] a, bottles monitored for substrate mineralization only. [d] Control refers to sterile control. [e] NC, no change.

TABLE 3. Amount of [^{14}C-UL]Benzene Detected as Gaseous End Products in Incubations of Aquifer Sediments from MI and CA

sample type	redox condition[a]	[^{14}C]benzene added (dpm)	$^{14}CO_2$ recovered (dpm)	$^{14}CH_4$ recovered (dpm)	^{14}C recovered as gas (%)
aquifer sediment from a national park, MI	M	3.50×10^6	1.48×10^6	1.39×10^6	82
	M control[a]	3.50×10^6	1.50×10^4	0	0.43
	S	9.17×10^6	7.17×10^6	ND[b]	78
	S control	8.20×10^6	9.10×10^3	ND	0.11
aquifer sediment from Seal Beach, CA	S	1.95×10^7	1.48×10^7	0	76
	S control	1.95×10^7	1.90×10^3	ND	0.01

[a] As in Table 2. [b] ND, not determined.

serum vials, which were sealed with thick butyl rubber stoppers. An anoxic slurry of poorly crystalline iron(III) oxide was added to the sediments to provide approximately 10 mmol L^{-1} additional Fe, as described previously (38). Benzene was added from an anoxic aqueous stock solution to provide an initial concentration of ca. 3 μM.

Analytical Techniques. Benzene removal from the various enrichments was monitored by gas (GC) or high-pressure liquid chromatography (HPLC) as indicated below. In experiments with OK or MI aquifer sediments and unlabeled benzene, loss of the compound was monitored by HPLC as previously described (42). In addition, methane was determined by GC as previously described (43). For [^{14}C]benzene studies with MI and CA sediments, the radiolabel was analyzed with an HPLC system equipped with an UV:in-line radio-isotope detector (Beckman Model LC 1801, 171 radioisotope detector, Fullerton, CA), with the mobile phase (60% aceto-nitrile, 40% sodium acetate of 50 mM) and the scintillation cocktail (Ready Flow-III, Beckman) flow rates at 0.5 mL min^{-1}. $^{14}CO_2$ was monitored by counting the radioactivity in alkali traps as described previously (42), and $^{14}CH_4$ was measured with a GC equipped with a gas proportional counter (27). Hydrogen sulfide production was determined spectropho-tometrically by the method of Fogo and Popowsky (44). In experiments with NY/NJ Harbor sediments, benzene loss was evaluated by GC—FID according to Coschigano et al. (45). As described previously, methane production was monitored by GC-TCD, sulfate and nitrate loss were determined by ion chromatography, and Fe^{2+} production was assayed by the ferrozine spectrophotometric method (41). Benzene removal

from Potomac River sediments was monitored by GC according to Lovley et al. (27).

Results and Discussion

This study demonstrates that microorganisms in enrichments established with sediments from a diversity of environments have the potential to degrade benzene under strict anoxic conditions. Despite differences in methodology and initial substrate concentration, anaerobic benzene metabolism was observed in sediments from aquifers, freshwater, and es-tuarine sources and under methanogenic, sulfate-reducing, and Fe(III)-reducing conditions (Table 2). These results confirm previous reports of anaerobic benzene utilization and extend the observations to other sediments. In addition, evidence for nearly complete conversion of [^{14}C]benzene to $^{14}CH_4$ and $^{14}CO_2$ are presented.

Previous studies have shown that benzene is degraded under methanogenic conditions, but the extent of mineral-ization was minor, with less than 6% of the initial amount of [^{14}C]benzene converted to $^{14}CO_2$ ($^{14}CH_4$ was not monitored) (23, 24). In contrast, in the results reported here, over 80% of the [^{14}C]benzene added to MI aquifer sediments was recovered as $^{14}CO_2$ and $^{14}CH_4$ (Table 3). The amount of $^{14}CO_2$ and $^{14}CH_4$ produced was 113% and 64%, respectively, of the theoretically expected values based on the Buswell equation (46). In the sterile controls, no $^{14}CH_4$ was noted, and less than 0.5% of the radiolabel added was recovered as $^{14}CO_2$.

Methane production was also observed when MI sedi-ments were incubated with 675 μM unlabeled benzene over 590 d (Figure 1). During this time, no intermediates of
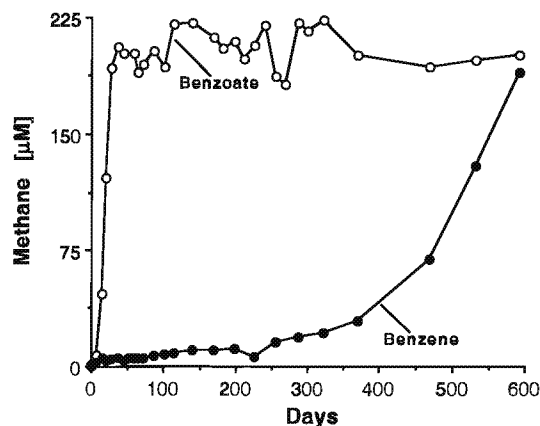
FIGURE 1. Methane production in aquifer sediments from Michigan, incubated under methanogenic conditions. Benzoate and benzene were supplied at a concentration of 50 ppm carbon. The curves are corrected for the amount of methane produced in substrate-unamended controls.

FIGURE 2. Loss of benzene under sulfate-reducing conditions in NY/NJ Harbor sediments. After an initial loss of approximately 125 μM benzene within 100 d, cultures were repeatedly fed with substrate over the next 80 d. Results are means of 3 replicates $\pm$ 1 SD and are corrected for abiotic losses in the autoclaved controls.

TABLE 4. Consumption of SO_4^{2-} during Degradation of Benzene in New York/New Jersey Harbor Sediments

benzene metabolized (mM)	SO_4^{2-} consumption (mM)		% of expected
	predicted[a]	measured[b]	
0.97 $\pm$ 0.02	3.63 $\pm$ 0.08	3.09 $\pm$ 0.55	85 $\pm$ 13

[a] Based on stoichiometry of 1 mol of benzene = 3.75 mol of SO_4^{2-}. The stoichiometric equation for the complete mineralization of benzene is

$$C_6H_6 + 3.75SO_4^{2-} + 3H_2O \rightarrow$$
$$6HCO_3^- + 1.875HS^- + 1.875H_2S + 0.375H^+$$

[b] Consumption of SO_4^{2-} in background control cultures (less than 0.1 mM within 195 d) subtracted.

benzene decomposition were detected by HPLC. After 600 d, when benzene was no longer detected in the benzene-amended incubations, the amount of methane produced was similar to the amount observed in the positive benzoate control. This is consistent with the Buswell equation (46), as both substrates were added at concentrations equivalent to 50 ppm carbon, and similar amounts of methane were expected to be produced in the benzene- and benzoate-amended bottles. Furthermore, the amount of methane produced in the benzene-amended bottles was 73% of that expected, assuming complete metabolism of benzene by the following stoichiometric equation: $C_6H_6 + 4.5H_2O \rightarrow 2.25CO_2 + 3.75CH_4$. The lower than expected values may be explained by incorporation of substrate into cellular material, adsorption of benzene to sediments or vessels, or loss from the incubations through some other means. These results clearly show, however, that benzene can be metabolized to yield large amounts of gaseous end products under strictly anoxic conditions. To confirm methanogenic benzene utilization in MI sediments, the slurries were reamended with 100 μM of [^{14}C]benzene after unlabeled benzene was no longer detected in the bottles. After 21 d, ^{14}CH$_4$ and ^{14}CO$_2$ were detected in the headspace, indicating that benzene ring cleavage and mineralization had occurred (data not shown).

Anaerobic benzene degradation was also observed in sulfate-reducing enrichments established with inocula from gasoline-contaminated aquifers in MI and Seal Beach, CA, and from a petroleum-contaminated estuarine site in NY/NJ Harbor (Table 3, Figure 2). Reports of benzene oxidation under sulfate-reducing conditions have been previously noted for slurries with aquifer (26), marine (27–30), and freshwater (31) sediments. The study presented here extends this metabolic potential to estuarine sediments and confirms a previous observation of benzene degradation in aquifer sediments from Seal Beach (26). In radiolabel studies with MI sediments, 78% of [^{14}C]benzene added was recovered as ^{14}CO$_2$ (Table 3), and a statistically significant amount of hydrogen sulfide was produced relative to the benzene-unamended controls (data not shown). Slurries established with CA aquifer sediments also exhibited mineralization of [^{14}C]benzene, with 76% of the label recovered as ^{14}CO$_2$ and no ^{14}CH$_4$ formation (Table 3). The loss of sulfate or the production of hydrogen sulfide, however, could not be discerned against background levels in substrate-unamended controls (data not shown). Benzene utilization was also observed in slurries with NY/NJ Harbor estuarine sediments, with degradation activity sustained upon numerous re-amendments of the substrate (Figure 2). Furthermore, the loss of sulfate was 85% of that expected for the complete
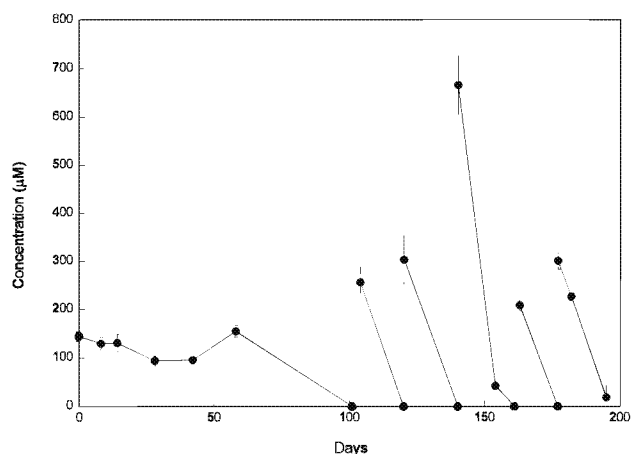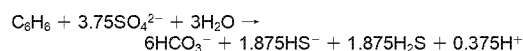
mineralization of benzene to CO_2, indicating that, in these sediments, benzene degradation was coupled to sulfate reduction (Table 4). This stoichiometry is similar to that obtained with benzene-adapted marine sediments (27), and comparable stoichiometries have been recently observed in sediments from a hydrocarbon seep (28) and benzene-adapted, sulfate-containing freshwater sediments (31).

In this study, benzene was not always degraded under methanogenic or sulfate-reducing conditions. Even after 3 years, benzene was recalcitrant in aquifer sediments impacted with landfill-leachate from Norman, OK (data not shown). It was initially thought that these results are due to high benzene concentrations (1.4–4.3 mM), and the microorganisms may have been adversely affected. On the other hand, benzene metabolism was not observed subsequently in these sediments even when a lower concentration (1.5 μM) of [^{14}C]-benzene was added (data not shown). A possible explanation is that benzene may not have been an important component of landfill-leachate contamination at the site and that the sediments were not enriched for benzene-degrading microorganisms. Flyvbjerg et al. (20) also observed no loss of benzene under sulfidogenic conditions within 7 months of incubation in mesocosms containing creosote-contaminated groundwater.

The absence of benzene-degrading microorganisms may be another factor affecting the lack of benzene metabolism (31). When aquifer sediment with no activity was inoculated with benzene-oxidizing microorganisms derived from other aquatic sediments, then rapid, sulfate-dependent benzene oxidation was noted. Thus, the source of the sediment inoculum and/or history of contamination may be important for microbial benzene degradation to occur.
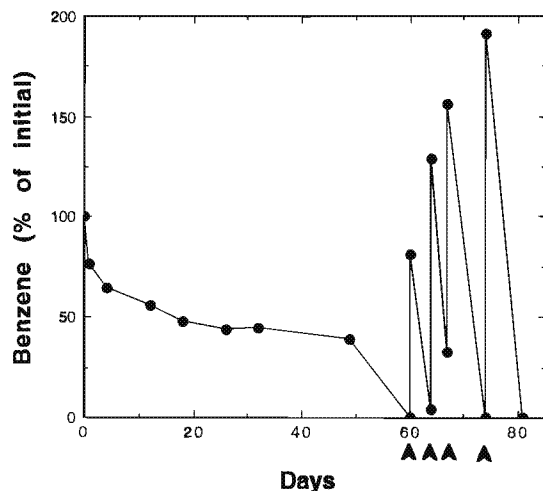
FIGURE 3. Benzene loss in iron(III) oxide-amended cultures established with Potomac River sediments. At day 0, the initial benzene concentration was 3 μM. The arrows indicate when benzene was depleted in the cultures. After an initial loss of 3 μM benzene within 60 d, cultures were re-fed with benzene over the ensuing 20 d.

Benzene loss under Fe(III)-reducing conditions was observed in Potomac River sediments in the absence of added Fe(III) chelators (Figure 3), suggesting that not all sediments require the addition of Fe(III) chelators. This was also shown with sediment from a petroleum-contaminated aquifer (47). Anaerobic benzene metabolism at the expense of Fe(III) reduction in aquifer material had been previousely reported (32, 33). In these reports, benzene loss occurred only when Fe(III) chelators such as EDTA and NTA were added, which makes Fe(III) more available for microbial reduction.

Microbial benzene loss was also noted in slurries established with NY/NJ Harbor estuarine sediments in which Fe-(III) was provided as a potential electron acceptor (Table 2). Benzene utilization, however, was not sustained upon re-feeding with the substrate. This may be because the sulfate contained in the original sediment inoculum was the electron acceptor for the initial benzene degradation observed, and once sulfate was depleted, benzene oxidation stopped. The initial sulfate concentration in the sediment slurries established with estuarine sediments was calculated to be approximately 1 mM. In a similar manner, benzene loss was observed in NY/NJ Harbor sediments to which carbonate was added to promote methanogenic conditions (Table 2), but the activity could not be sustained with re-feeding of the substrate. Methane production was noted in benzene-amended bottles; however, the amount produced was similar to that in the benzene-unamended background controls. These results are also consistent with the likelihood that benzene utilization is coupled to the reduction of sulfate included in the inoculum.

There was no metabolism of benzene under denitrifying conditions in cultures established with sediment from a gasoline-contaminated aquifer (MI) or a petroleum-contaminated estuary (NY) within 530 and 210 d, respectively (Table 2). Nitrate-dependent benzene uptake in anoxic aquifer sediments has been reported (34, 35), although in further experiments using the same sediment source, benzene degradation under denitrifying conditions was not observed (17). Other field and laboratory studies have also found that benzene was not metabolized under strictly denitrifying conditions (11–13).

In this study, benzene utilization proceeded relatively rapidly after an initial lag period of 50–420 d (Table 2). There could be a number of reasons to account for the variable length of time where biodegradation does not occur to an appreciable degree (48, 49). These include the time required for an initially small population size to grow sufficiently large to achieve detectable degradation rates, the lack of essential nutrients, and the need for genetic alterations (i.e., mutation, gene exchange, or rearrangement) prior to the onset of metabolism. Although the mechanism(s) involved in each of the cases presented is not clear, it should be noted that benzene metabolism was observed in enrichments established from a variety of sediments, ranging from freshwater aquifer material to fine-grained, estuarine muds, at different initial benzene starting concentrations, and under a wide variety of electron-accepting conditions.

In summary, the results demonstrate that the potential for microbial benzene degradation exists under a number of different reducing conditions. This is in contrast with many earlier studies which show that benzene degradation is minimal in the absence of oxygen. Although factors that may account for these differences are not clear, possible explanations for our observations include the realization that long incubation times may be necessary for activity to be evident, the addition of benzene as the sole substrate in order to avoid preferential utilization of other aromatic hydrocarbons (26), and the sampling of sediments that are known or suspected to be contaminated with petroleum compounds. Now that the potential for anaerobic benzene degradation has been demonstrated in a diversity of sediment types and is more widespread than previously thought, it will be important to determine the significance of this process in removing benzene from contaminated environments.

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